REMARKS

Claims 2-5, 8-10, 13-16, 19-21 and 24-35 were pending. Claims 13-16, 19-21, and 24-35 are cancelled by the present amendment without prejudice to Applicants' right to prosecute their subject matter in the present application and in related applications. Claim 2 is currently amended. Accordingly, claims 2-5 and 8-10 are pending and presented for examination.

Claim 2 is amended to recite a method for reducing sepsis-associated lethality in a mammal with sepsis and to remove unnecessary words from the claim. Support for the amendment to claim 2 is found in the originally-filed application at least, for example, at page 9.

Applicants submit that the amendment introduces no new matter into the application.

Claim rejections : Written Description

All pending claims stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Specifically, the Office action alleges that there is no written description permitting TCF-II to be administered prior to the onset of sepsis. To advance prosecution, Applicants have amended the claims without prejudice to remove the reference to administration prior to the onset of sepsis. As amended, the claims recite a method for reducing sepsis-associated lethality in a mammal with sepsis. Accordingly, Applicants submit that the amended claims comply fully with the written description requirement of 35 U.S.C. § 112 and respectfully request reconsideration and withdrawal of the rejection.

Claim rejections : Enablement

All pending claims stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. Specifically, the Office action alleges that the specification does not enable a "method for reducing sepsis-

associated lethality in a *mammal that develops sepsis*" (emphasis in Office action) because the specification allegedly does not enable the predictable and reproducible identification of "those mammals that will develop sepsis (i.e. in the future)" (Office action, page 5). To advance prosecution, Applicants have amended the claims without prejudice to remove the reference to "a mammal that develops sepsis." As amended, the claims relate to a method for reducing sepsis-associated lethality in a mammal with sepsis. Accordingly, Applicants submit that the amended claims comport fully with the enablement requirement of 35 U.S.C. § 112, first paragraph, and respectfully request reconsideration and withdrawal of this rejection.

Claim rejections : Definiteness

Claims 16, 24, 28, 30 and 35 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. To promote prosecution, Applicants have cancelled each of claims 16, 24, 28, 30 and 35 without prejudice. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections.

Claim rejections : Anticipation

Claims 13-16, 19-21, 31 and 33 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Canadian patent application 2100720. To promote prosecution, Applicants have cancelled each of claims 13-16, 19-21, 31 and 33 without prejudice. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections.

All pending claims stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by U.S. Patent No. 5,714,461 ("the '461 patent"). Applicants traverse this rejection to the extent it is maintained over the amended claims.

The '461 patent does not anticipate the pending claims. "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference" (MPEP § 2131). As amended, the

pending claims relate to a method for reducing sepsis-associated lethality in a mammal with sepsis. The method includes administering an amount of isolated or purified TCF-II effective to reduce sepsis-associated lethality. The '461 patent, while describing administration of TCF-II to a subject afflicted with disseminated intravascular coagulation (DIC) and teaching that DIC accompanies various basal diseases including malignant tumors, leukemia, sepsis, infectious diseases, obstetric diseases and shock (paragraph bridging columns 1 and 2), *does not describe administering TCF-II to a mammal with sepsis in an amount effective to reduce sepsis-associated lethality.* The '461 patent teaches administering TCF-II to rats after a "70% hepatectomy to prepare a pathologic model of liver damage and DIC symptoms" (col. 7, lines 57-58), not to rats identified as septic. Because the '461 patent does not describe administering TCF-II to a mammal with sepsis in an amount effective to reduce sepsis-associated lethality, the '461 patent cannot anticipate the pending claims.

Applicants wish to make clear that sepsis and DIC are recognized as separate conditions in the art. As indicated in the '461 patent, *DIC can be caused by a variety of non-septic conditions*. These teachings of the '461 patent are consistent with the teachings of Penner (1998) Seminars in Thrombosis and Hemostasis, 24:1, 45-52, a copy of which is attached as Exhibit A, which provides that "[d]isseminated intravascular coagulation (DIC) and associated multi-organ failure are serious and often terminal events of a variety of non-septic conditions" (Penner, abstract). According to Table 1 of Penner, these non-septic conditions include trauma (impact, surgical, burn injuries), malignancy (pancreas, prostate, leukemia (acute promyelocytic, acute myeloblastic)), obstetric conditions (placenta abruptio, placenta previa, amniotic fluid embolism, dead fetus syndrome, saline induced abortions, retained placenta, preeclampsia), immune disorders (lupus erythematosus, thrombotic thrombocytopenic purpura, vacsculitis autoimmune hemolysis, incompatible blood transfusions), vascular conditions (giant hemangiomas, aortic aneurysms, prosthetic devices (balloon assist device, arterial grafts), envenomation (insects and snake bites), and chronic inflammatory disorders (Crohn's disease, sarcoidosis, ulcerative colitis).

Thus, DIC can *arise* through non-septic causes. Similarly, DIC *exists* in the absence of sepsis. For example, Yajima *et al.* (1989), Gastroenterologia Japonica, 24(3):262-269, a copy of which is attached as Exhibit B, describes cirrhotic patients with DIC as non-septic: "Endotoxin-specific [limulus colorimetric test] did not detect any endotoxin activity throughout the courses of any of the cases. Consequently, endotoxins detected in this study were all non-septic endotoxins according to our definition" (Yajima, p. 263).

In the '461 patent, TCF-II is administered to rats with 70% hepatectomies, not to rats with sepsis. The '461 patent teaches administering TCF-II to a subject afflicted with DIC and teaches that sepsis is a cause and indication, but does not describe administering TCF-II to a mammal with sepsis. Because the '461 patent does not describe each and every element of the pending claims, the '461 patent cannot anticipate the pending claims. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

Claims 2-5 and 8-10 are pending and presented for consideration. The Examiner is encouraged to contact the undersigned to discuss any outstanding issues.

Respectfully submitted,

Brian A. Fairchild, Ph.D.
Attorney for Applicants
Testa, Hurwitz & Thibeault, LLP
High Street Tower
125 High Street
Boston, MA 02110

Date: October 25, 2004
Reg. No. 48,645
Tel. No.: (617) 248-7697
Fax No.: (617) 248-7100

Disseminated Intravascular Coagulation in Patients with Multiple Organ Failure of Non-Septic Origin

JOHN A. PENNER, M.D.

ABSTRACT **Disseminated intravascular coagulation (DIC) and associated multi-organ failure are serious and often terminal events of a variety of non-septic conditions. For the most part, these conditions are a result of tissue factor (thromboplastin) release from damaged tissues creating situations that favor thrombin formation. Thrombin's role in this process is critical and serves to induce the coagulopathy, as well as many of the other aspects of inflammation that contribute to the associated morbidity and mortality.**

Clinical disorders giving rise to DIC fall into categories of trauma, obstetrical complications, malignancies and a variety of inflammatory conditions. Diagnostic patterns for these disorders are well established with an expected decrease in platelets and fibrinogen, as well as antithrombin III, in addition to elevated levels of thrombin-antithrombin III complex, prothrombin fragment 1 + 2, and D-dimer; all of which serve to identify the hypercoagulable state.

Management of these coagulopathies requires attention to the bleeding diathesis and the ongoing thrombotic complication. Supportive therapy usually is required to provide hemostasis. However, control of the coagulopathy is of equal importance and requires not only early intervention, but also administration of sufficient antithrombotic agents to reduce thrombin's ability to consume coagulation factors, as well as to stimulate inflammatory processes. Heparin has been employed effectively in many of these situations, but suffers from its potential to induce hemorrhage. Antithrombin III concentrate, however, is devoid of this risk and provides a unique alternative that has had a limited, but effective record of benefits. Further proof of its efficacy in multi-organ failure disorders is awaited.

Key words: **Disseminated intravascular coagulation (DIC), trauma, antithrombin III concentrate, multiple organ failure, acute respiratory distress syndrome, microvascular thrombosis.**

Coagulopathies appearing as hemorrhagic as well as thrombotic disorders are a frequent finding in many critically ill patients and are associated, if not often responsible, for multi-organ failure syndromes (MOFS) and their fatal consequences. This process, termed disseminated intervascular coagulation (DIC), represents an exaggeration of what can be considered to be a constant, sometimes irregular, state of intervascular coagulation that normally proceeds in a controlled fashion, permitting a compensated turnover of the many factors involved in hemostasis. Such exaggerated, or pathologic disturbances in hemostasis are defined by a dramatic consumption of coagulation factors with subsequent depletion to levels which no longer support hemostasis. Therefore, the bleeding that commences becomes uncontrollable and life threatening. Associated with this process is a phase of hypercoagulability that, paradoxically, produces thrombotic events when the appropriate conditions such as stasis are present. Thus both bleeding and thrombosis combine to produce an incidence of morbidity and mortality that is not easily affected by our interventions. Sepsis is well established as an etiologic factor for intravascular coagulation with the identification of the responsible polysaccharide remnants, residuals of gram negative bacteria. For the most part, the non-septic basis of this disorder is tissue factor derived and was established initially from an investigation of the clinical complications associated with obstetrical disorders.

HISTORICAL BACKGROUND

The hemorrhagic complications of amniotic fluid embolism, placenta previa, and placenta abruptio were recognized for many years and were believed to be due to a condition called primary fibrinolysis. The rapid liquefaction of clotted blood from such patients attested to

From the Department of Medicine, Michigan State University College of Human Medicine, East Lansing, Michigan.

Reprint requests: Dr. Penner, Department of Medicine, Michigan State University, B-338 Clinical Center, East Lansing, MI 48824-1315.

the fact that clot lysis was responsible, although, the fibrinogen residual in such blood was insufficient to produce more than a thin film which easily could be dislodged by tilting the tube of clotted blood that often was left taped to the end of the patient's bed, a common practice at that time.

Dr. Charles Schneider (an obstetrician and gynecologist), working in the laboratory of Professor Walter Seegers in the Department of Physiology at Wayne University in Detroit, provided some of the first insights as to the nature of this process. Schneider, aware of this hemorrhagic complication in his clinical practice, proceeded to develop an experiment whereby extracts of placental tissue were infused intravenously in rabbits who subsequently developed a hemorrhagic diathesis within several hours after the administration. The pattern of bleeding and the striking decrease in clotting factors that occurred following the infusion were similar to what Schneider had observed in many of his patients. In a series of papers, Schneider and Seegers[1,2] elaborated on the mechanisms involved in these disorders describing experiments easily duplicated in the laboratory by classical techniques. This position was supported by Ratnoff and colleagues in their review of the hemorrhagic states of pregnancy.[3] That such events could be happening under differing clinical conditions also was realized, as the defining laboratory assays were applied to bleeding patients presenting with a variety of critical illnesses.

EXPERIMENTAL STUDIES

The classical experiments can be considered as follows: Tissue factor was obtained as filtered saline extracts of ground or macerated organs (i.e., lungs, placenta, uterus, etc.); when it is infused very rapidly into a rabbit, it produced immediate death of the animal. The cause of death, thrombosis of the pulmonary artery extending from the right ventricle, can be demonstrated at necropsy. On the other hand, the same dose of tissue factor extract administered slowly over several hours does not appear to affect the animal to any measurable degree. Apparently, the natural occurring major inhibitors of coagulation; tissue factor pathway inhibitor (TFPI), antithrombin III (AT III), heparin cofactor II, (HCII), and proteins C (PC) and S (PS) are sufficient to prevent progressive thrombogenesis. A more rapid intravenous administration over an intermediate period (perhaps 20 min) of the same amount of tissue extract produces little effect immediately. Surprisingly, however, in several hours pulmonary bleeding is experienced and the animal dies, primarily as a result of hemorrhage into the lungs and to a lesser extent into other organs. The basis for the bleeding manifestations appearing in this last experiment is easily identified by measurements of the various clotting factors, noting a rapid decline of fibrinogen as well as platelets, and significant decreases in Factors V and VIII, Factor XIII, and the physiologic inhibitors AT III, PC and PS over the 2 h period. A lesser degree of decline is seen in the vitamin K dependent protease precursors (Factors II, VII, IX and X) as expected, recognizing the central role of thrombin, which exerts its effect at very low concentrations, and thus does not require activation or loss of large quantities of these protease promoters. Similar experimental induction of intravascular coagulation has been demonstrated with the administration of thrombin itself, as well as activated vitamin K dependent factors and several snake venoms.[4]

An additional experiment that sheds further light on the clinical complications associated with DIC relates to the presence of organ-specific venous stasis that occurs with severe hypotension and shock. Under such conditions, renal necrosis often is seen as a result of microthrombi induced ischemia developing as renal blood flow is reduced. The experiment requires the same infusion of an instigating substance, such as tissue factor, in addition to a small amount of epinephrine sufficient to induce constriction of the renal vessels (similar to that seen under conditions of shock). Following these interventions, urine output declines strikingly, resulting in anuria, while blood serum values of urea nitrogen and creatinine increase. At necropsy, the kidneys are ischemic and microthrombi can be found diffusely throughout the renal pelvis. Thus the hemorrhagic and thrombotic manifestations of disseminated intravascular coagulation can be induced in much the same manner as seen when these complications arise in clinical practice.

LABORATORY ASSESSMENT

If the process of DIC is dissected, it represents (in simple terms) the conversion of plasma to serum. When conditions are favorable, this process is exhibited in vivo. Platelets gradually disappear as they aggregate and adhere to a vascular bed that has been damaged by thrombin, and by substances elaborated from the circulating neutrophils and macrophages attracted to the injured areas. Fibrinogen, an acute phase reactant, although often increased in critical illnesses, rapidly declines as it is converted to fibrin, and subsequently, layers out along the vascular bed in the fast flowing blood of the major arteries. Factors V and VIII are reduced as well as thrombin and transformed to their active forms, V_a and $VIII_a$, which then combines with thrombomodulin activated PC, which, with PS, inactivates them. In severe cases of DIC, the laboratory pattern as we generally assess it in clinical situations, provides us with prolongation of the prothrombin time (PT) and activated partial thromboplastin time (APTT), as a result of diminished fibrinogen as well as Factors V and VIII. A decline in fibrinogen and platelets and the inhibitors AT III and PC and PS contribute additional evidence to support the diagnosis of DIC. In as much as this is a dynamic state in clinical situations, the changes in these parameters are exhibited over time in a manner

similar to that seen in the test tube with plasma gradually being converted to serum (serum no longer coagulable, and therefore, unsupporting of hemostasis). The hemorrhagic aspects of the disorder then become prominent as the consumption of the coagulation factors proceeds. With the introduction of hypotension or a shock-like state, thrombotic events will be manifested as was described experimentally. Organ failure usually begins with renal ischemia, eventually producing cardiac and hepatic failure. The acute respiratory distress syndrome (ARDS) may supervene, as the inflammatory process spreads and complications arise from the release of a variety of mediators (i.e., intercellular adhesion molecule (I-CAM), interleukin 1 (IL1), platelet activating factor (PAF), tissue necrosis factor (TNF), etc. This insult to pulmonary function often is the final event, if the patient does not succumb to hemorrhage.

FORMS OF CLINICAL DIC

Non-septic clinical disorders include trauma resulting from impact, surgical, and burn injuries, as well as malignancies and a variety of obstetrical and inflammatory disorders. A partial list is provided in Table 1. In almost all instances, tissue factor provides the stimulus to initiate thrombin formation with subsequent conversion of fibrinogen to fibrin, followed by activation of the fibrinolytic system which results in gradual defibrinogenation and leads to the bleeding diathesis seen in the experimental model.

TRAUMA

Trauma in all its forms realizes the effects of tissue damage, however, acute hemorrhage often is a result of the initial injury and if massive, may lead to hypotension and a shock-like state, fulfilling the conditions for renal ischemia and thrombosis in addition to hemorrhagic manifestations.

A number of studies have been presented describing the development of progressive coagulopathies as a result of severe impact injuries from accidents involving motor vehicles, falling from great heights, and bullet and multiple knife wounds. Owings and Gosselin[5] reviewed 157 such cases appearing in their critical care unit, and observed a decline in antithrombin activity to less than 80% in 95 of their patients, and less than 50% in 24. Nine of the patients developed DIC, most with ARDS, as well as thromboembolic complications. Gando et al.[6] reported 40 patients, 15 with DIC, noting increased D-dimer as well as thrombin antithrombin (TAT) complex levels in all patients, although values were somewhat higher in those with DIC. Tissue plasminogen activator antigen was only increased in patients with DIC. Other investigators,[7,8] have reported similar experience, noting a decline in AT III levels consistent with the severity of injury score (ISS), and particularly with the presence of shock. All with high ISS displayed advanced DIC with increased fibrinolytic activity.[9,10] Those with hypotension on arrival at the hospital or at the time of injury, demonstrated lower AT III, PC, and antiplasmin levels, as well as higher concentrations of TAT and D-dimer values. Gando et al.[10] in a well documented second paper described 58 trauma patients, 22 with DIC.[10] The DIC patients exhibited a higher ISS and, as expected, had a higher mortality rate, 59% versus 13.8%. Tumor necrosis factor alpha (TNF_a), interleukin 1 beta (IL-1), tissue plasminogen activator inhibitor (PA-1) increase was most consistently associated with fatal outcome, while DIC was a predictor of ARDS and multi-organ failure (MOD).

Blunt trauma to the head is a particularly effective initiator of events that lead to DIC. The brain, being rich in phospholipids, is capable of supporting a coagulopathy as the damaged tissues leak these products into the circulation. Kearny et al.[11] evaluated outcomes in 36 patients with severe head injuries resulting in a 50% mortality. These patients' assigned Glascow Coma Scores (GCS) score of less than 9 were compared to five patients undergoing elective neurosurgery. All trauma patients demonstrated forms of coagulopathy that were rated on a DIC scale of 0 to 3. Survivors had lower DIC scores and had less severe injuries as expected. The nonsurvivors had lower fibrinogen levels and more prolonged PT and APTT values, lower AT III, PC and PS, and higher D-dimer levels. Neurosurgical control patients had a slight D-dimer elevation and decreased APTT values. Several similar studies of patients with blunt brain injuries have observed severe depletion of fibrinogen; elevated F 1+2 and TAT within the first hours of injury correlating with the severity of the injury.[12,13]

TABLE 1. DIC Associated Conditions (Non-Septic)

Trauma	Impact, surgical, burn injuries
Malignancy	Pancreas, prostate, leukemia (acute promyelocytic, acute myeloblastic)
Obstetrics	Placenta abruptio, placenta previa, amniotic fluid embolism, dead fetus syndrome, saline induced abortions, retained placenta, preeclampsia
Immune Disorders	Lupus erythematosus, thrombotic thrombocytopenic purpura, vasculitis autoimmune hemolysis, incompatible blood transfusions
Vascular	Giant hemangiomas, aortic aneurysms, prosthetic devices (balloon assist device, arterial grafts)
Envenomation	Insects and snake bites
Chronic Inflammatory Disorders	Crohn's disease, sarcoidosis, ulcerative colitis

BURN INJURIES

Thermal trauma also has a recognized incidence of DIC, resulting from release of tissue factor, IL-1 beta, TNF alpha. Often significant hemolysis occurs with such injuries giving rise to the release of erythrocyte platelet membrane lipids which help to sustain the coagulopathy. Some 60 patients admitted to the Loyola University Burn Center, as reported by Walenga et al. exhibited various degrees of coagulopathy related to the severity of injury.[14] Prolonged PT, APTT, decreased AT III, PC and PS were observed in their cases. The possibility that the dilutional therapeutic treatment may have influenced these results was not supported by the fact that elevated levels of TAT, t-PA, and plasminogen activator inhibitor (PAI), as well as D-dimer were found. In a group of 25 severely burned children, Neely et al.[15] noted that AT III, alpha 2 antiplasmin and plasminogen were decreased while neutrophil elastase was increased.[15] The uninhibited proteolytic activity correlated with the percent of the total body surface area damaged in their study.

A consistent theme, no matter what the cause of trauma, appears to be a direct relationship of the coagulopathy to the severity of the injury. In addition, it is evident that early signs of DIC exist with the injury and proceed rapidly with the first few hours, unless intervention is offered (as discussed later). Once DIC is fully established the associated complications of multi-organ failure often lead rapidly to a fatal outcome.

OBSTETRICAL DISORDERS

The clinical complications faced by obstetricians served to stimulate interest in consumption coagulopathies, as described, with recognition of DIC in many of these conditions: amniotic fluid embolism, placenta previa, dead fetus syndrome, retained placenta, and saline induced abortion. Even preeclampsia, a relatively common occurrence in pregnancy, manifests activation of the coagulation system as a result of endothelial damage, a concept supported by reports attesting to an increase in circulating fibronectin and thrombomodulin, both indicators of endothelial damage.[16,17]

Amniotic fluid embolism occurs primarily during labor (80%), and is more frequently seen in the older multiparous patients, particularly those undergoing an induced delivery. Approximately 10% of the maternal deaths in the United States were reported some years ago to result from this condition with 25% in the first hour, while fetal death or distress occurred in 50% before the appearance of the symptoms.[18] The presence of mucus or meconium from the stressed fetus is believed to potentiate the thrombogenicity of the amniotic fluid once it has entered the maternal circulation, eventually, leading to obstruction and thrombosis of the pulmonary bed, right-sided heart failure, metabolic acidosis, endothelial cell injury, and a terminal form of DIC.

Coagulopathies occurring in all of these complications, placenta previa, abruptio, and dead fetus syndrome, primarily are a result of tissue factor released in the maternal blood stream, although massive blood loss, and shock in placental abruption contributes to the coagulopathy.[19] This latter condition occurs in 1% of all pregnancies, and accounts for 35% of perinatal mortality. Retained placenta is also a common denominator for the postpartum appearance of DIC as the placental remnants necrose.

In most instances, the elimination of the fetus or the residual placenta alleviates the disorder and relieves the coagulopathy. In several instances, however, curettage has failed to dislodge remaining portions of placenta and hysterectomy has been necessary.

MALIGNANCIES IN DIC

Neoplasms of various origins often are complicated by and may present with the hemorrhagic complications of DIC. The most common example is seen with acute promyelocytic leukemia (APL), a condition in which substances released from the leukemic promyelocytes initiates activation of the coagulation as well as the fibrinolytic system.[20] Kario et al.[21] observed elevated levels of thrombin antithrombin complex circulating in patients with APL, however, they also identified even greater increases in plasmin antiplasmin complexes (PAP) attesting to the activation of both systems with resulting DIC and hemorrhage.[21] Other forms of leukemia also are associated with DIC, although to a lesser extent and more often following chemotherapy with the resulting lysis of leukemic cells and release of myeloperoxidase, elastase and other enzymes that create endothelial damage.[22]

For the most part, malignancies produce activation of the coagulation system as they outgrow their blood supply and become necrotic. The pattern, similar to trauma, involves tissue factor release. Lung, liver, and prostate cancers have this tendency. Pancreatic cancers, however, with their potential for release of a variety of proteases are unique in this regard and often present with multiple thrombotic events as well as consumption coagulopathies.[23]

These situations can be puzzling when the malignancy is not obvious. For example, I specifically recall a patient who presented with hemorrhagic manifestations and a pattern of DIC with decreased levels of fibrinogen, antithrombin, and platelets. Eventually, biopsy of an enlarged tender lymph node demonstrated Hodgkin's disease characterized by the presence of significant areas of necrosis. The coagulopathy disappeared following the administration of chemotherapy.

MISCELLANEOUS INFLAMMATORY DISORDERS

Damage to organs and tissues from a variety of causes provides the opportunity for the development of a hypercoagulable state and compensated or decompensated forms of intravascular coagulation. Sarcoidosis, Crohn's disease, ulcerative colitis, and many of the collagen vascular disorders such as lupus erythematosus have demonstrated this propensity.[24-26] DIC also is seen in the end stage of the thrombotic thrombocytopenic purpura, and hemolytic uremic syndromes,[27] and may accompany severe liver damage caused by toxins such as methyl alcohol ingestion or by viruses which induce fulminant forms of hepatitis.[28] Even prolonged conditions of acidosis have lead to sufficient endothelial damage to initiate and promote intravascular coagulation,[29] while massive hemolysis from transfusion reactions or immune disorders provide enough membrane phospholipid to create DIC.[30] All of these disorders contribute the necessary elements to maintain a heightened state of coagulation that extends beyond the area of localized organ damage.

CHRONIC DIC

Although most of our clinical experience reflects acute and sporadic forms of DIC, chronic, partially compensated DIC has been recognized in several vascular disorders. The Kasabach-Merritt syndrome or giant hemangioma has a well described coagulopathy with activation and increased turnover of platelets.[31] Exposure to turbulent flow in an inadequate vascular bed activates platelets and creates sufficient thrombin to cause depletion of fibrinogen. A number of cases of aortic aneurysms have been reported with similar findings.[32] The latter have had demonstrable improvement with administration of anticoagulants or repair of the aneurysm.

THERAPEUTIC INTERVENTIONS

Attempts to manage disseminated intervascular coagulation and its associated complications have varied, recognizing the need to control bleeding resulting from the coagulopathy, as well as from the underlying cause (i.e., trauma, pregnancy, neoplasia, etc.). Our therapeutic efforts to control this process have been to reduce the impact of thrombin, as well as its promoters. In these situations, it is apparent that the inhibitory systems that control coagulation in vivo have been overwhelmed, and the necessity of reducing thrombin's many effects and its evolution are paramount. There is no doubt that the underlying clinical conditions responsible for thrombin's evolution will play a major role in the patient's survival; however, the coagulopathy itself is often life threatening and must be managed while awaiting improvement in the initiating circumstances.

With the exception of emptying the uterus in the obstetrical disorders and administering chemotherapy in responsive malignancies, it would seem evident that prevention of thrombin activation and neutralization of its activity should be offered if the underlying cause for the coagulopathy cannot be eliminated rapidly. Several effective antithrombotic agents are available for this purpose and more will be approved for therapeutic use in the future. In the meantime, the anticoagulant heparin has assumed a significant role in the management of the condition through its ability to enhance the physiologic antithrombin activity and, thus, inactivate thrombin as well as proteases such as factors X_a and IX_a, and to a lesser extent factors XI_a and VII_a which promote thrombin formation. Heparin, although catalyzing antithrombin's rate of thrombin inactivation by a thousand-fold, is still dependent upon the presence of substantial amounts of antithrombin to exert an effect. Unfortunately, AT III present in circulating plasma declines rapidly after it is complexed with thrombin and other proteases, and may be removed from circulation at a rate that cannot be compensated by an increased production from the liver. Concentrates of AT III have been produced for many years, and are now readily available for approved therapeutic use in patients with congenital deficiencies. The obvious and apparent benefit anticipated by administration of AT III in acquired deficiencies, however, has been substantiated by only a few experimental studies which demonstrated significant clinical improvement. Thus, efficacy must be judged on the basis of animal experiments and from results of several clinical investigations in human subjects now being carried out as multi-institutional studies. At the outset, however, it is necessary to recognize that with the consumption of the coagulation factors, a hemorrhagic diathesis is present and many patients are in an active bleeding state when seen. As a result, initially it is necessary to control bleeding by improving hemostasis and replacing the necessary coagulation factors: platelets, fibrinogen, Factors V, VIII, and XIII.

HEMOSTATIC SUPPLEMENTATION

Coagulation factors consumed by the severe, decompensated form of DIC process can be effectively replaced to restore hemostasis, and thus permit the use of anticoagulants to blunt the process. In as much as thrombocytopenia is one of the major concerns, administration of *platelet concentrates* can be effective by not only restoring platelet numbers, but also by providing additional amounts of factor V. Approximately 50 ml of plasma is available in each unit of platelets, and thus other plasma factors are provided as well. It should be noted that with the coagulopathy, to control bleeding, the platelet numbers need to be sufficiently increased but

not excessively increased, which would contribute to the thrombogenic process. Generally, 6 U of platelet concentrate will increase circulating platelets in an adult by approximately 40 $\times 10^9$/L. Additional units can be offered, as needed, at 4–6 h intervals. *Cryoprecipitate* provides the only source of fibrinogen approximately 200 mg per bag. Thus, 10 bags can contribute 2 g of fibrinogen to the circulation, an ample amount to increase circulating levels. Cryoprecipitate also contains Factor VIII, Factor XIII, von Willebrand factor, and fibronectin, all of which improve hemostasis. In the past, plasma has been the mainstay for supplementing hemostasis; however, despite its content of clotting factors, it must be administered in large quantities, at least 1 liter for an average adult, a volume often limited by the patient's cardiac status and risk of congestive heart failure. The use of the platelet concentrates and cryoprecipitate are to be recommended over plasma.

ANTICOAGULANT THERAPY

The means to control or dampen the progressing coagulopathy also are at hand. Heparin and AT III concentrate have been employed in a limited fashion in experimental models and in clinical studies (see additional articles in this journal). The effect of PC concentrates, as well as newer antiplatelet agents remains to be evaluated, although there has been some experience with Dextran and with the antithrombin, hirudin.

HEPARIN THERAPY

Animal experiments[33] have demonstrated heparin's ability to abort endotoxin or tissue factor induced DIC, if administered immediately after the initiating factor. Clinical experience would support the fact that heparin, at least in low dosage, is not deleterious in these situations, and in many instances would appear beneficial by slowing the rate of thrombin formation allowing reconstitution of hemostasis to the point that bleeding is controlled without supplementation of clotting factors and further thrombotic events are averted.[34,35] Unfortunately, there is a risk of promoting further hemorrhage if hemostasis has not been restored and thus administration often is tentative and necessarily delayed. Furthermore, the complexity of the DIC process, as well as the variability of cause prevents a meaningful measurement of outcome once the intervention has been applied. Clinical studies, therefore, have not provided adequate data to construct a clear-cut recommendation for heparin administration. A consensus of hematologists with experience is this area, I believe, would support the use of heparin, or an alternative to manage the coagulopathy, once bleeding has been controlled while awaiting correction of the underlying cause. A gradual approach initially employing low or prophylactic doses while restoring coagulation factors allows progression of heparin dosing to therapeutic levels once hemostasis has been achieved. Of course, heparin's efficacy is dependent upon adequate amounts of antithrombin for its ability to exert a catalytic effect. Therefore, antithrombin support may be a necessary requirement to achieve therapeutic benefits from heparin administration.

ANTITHROMBIN III IN THE MANAGEMENT OF DIC

A number of experimental DIC models were evaluated with respect to the efficacy of AT III in controlling the process. Experiences with sepsis models using lipopolysaccharides are well known, however, several animal models also were developed using prothrombin complex concentrates containing activated factors Factor X_a, IX_a, and VII_a[36] as the initiator of DIC. Initially, testing was undertaken to demonstrate the thrombogenicity of these products. Introduction of AT III concentrate was employed to determine efficacy of this agent in preventing a consumption coagulopathy, shown in Figure 1. As depicted, AT III concentrates administered following infusions of these proteases significantly limited the consumption of clotting factors preventing the thrombocy-

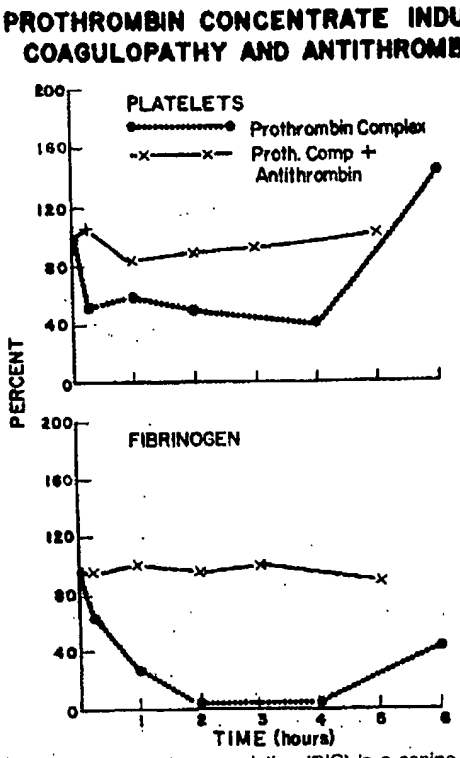

FIG. 1. Intravascular coagulation (DIC) in a canine model induced by protease containing prothrombin concentrate: Effect of AT III on circulating platelets and fibrinogen.

topenia and hypofibrinogenemia. Similar studies have been undertaken in the sepsis model with demonstration of dramatic improvement in mortality in rats, as well as baboons when very large doses of AT III were given in concert with the phospholipid initiator[37] (see also Dickneite in this issue).

Clinical studies in humans have been limited,[33,38,39] although, there is a wealth of information noting the occurrence of antithrombin deficiency in patients with disseminated intravascular coagulation resulting from trauma, malignancy, and severe hepatocellular disease as described. In most such studies, AT III activity has been found to be directly related to the severity of initiating disorder and inversely related to survival, with the prognosis being poorest in those patients with lowest circulating levels of antithrombin. Generally, trauma is found to produce a decrease in AT III levels, particularly during the first few hours after injury, whether due to impact, blunt, or penetrating trauma, or even burn injuries. These identifiable decreases in antithrombin levels are consistent with other measures of hypercoagulability and signs of inflammation. Therapeutic intervention with antithrombin concentrates are now beginning to be published. Jochum et al. reported a prospective placebo controlled randomized study of 40 patients.[40] The antithrombin concentrate was administered within 6 h of trauma. The patients experienced no side effects, especially no bleeding complications. Attempts were made to maintain plasma levels of antithrombin at 150% of normal, over a 3 to 4 day period. A mean dose of 21,000 U of antithrombin was administered per patient. Improvement in pulmonary function was identified, as measured by pulmonary artery oxygen. There was a significant reduction in artificial ventilation time of 23 days in the control group to 11 days in the antithrombin treated group. Intensive care unit days also were decreased from an average of 29 in the control to 13 days in the treatment group. Mortality figures were not significantly different in these poly-trauma patients. Other parameters measured, also demonstrated improvement including a reduction in soluble thrombomodulin, an endothelial cell receptor for thrombin.

A significant improvement in burn injury was reported by Kowal-Vern and colleagues who observed a reduction in graft failure, more rapid wound healing, and a shorter hospital course in several patients with severe thermal injury who received large amounts of antithrombin shortly after their admission.[41]

The efficacy of AT III in these studies was dependent on the time of administration after initiation of the process and the dose of antithrombin administered. If AT III was administered at the same time as induction or within a few hours after, it was possible to significantly improve outcome. However, the dose of AT III was found to be critical in order to be effective and was required in large quantities to obtain a pharmacologic rather than a physiologic level.

Several other prospective trials of AT in patients with DIC are in progress and have not been completed. From our animal experimental data, however, it can be anticipated that antithrombin will have a favorable impact on the coagulopathy, as well as the associated complications.

OTHER THERAPEUTIC AGENTS

A number of other agents that may have therapeutic implications in this disorder have been employed or are in the process of development.[34] Antiplatelet agents, for example, by reducing platelet activation occurring as a result of exposure to damaged endothelium have been used extensively and include aspirin, Dextran 70, and more recently, Tyclopidin, and platelet glycoprotein II_b/III_a inhibitors. Concentrates of PC and PS, as well as heparin cofactor II also may contribute to the control of the coagulopathy while argatroban and hirudin, two new experimental antithrombins should be on the market shortly. All of these agents have variable capacities to reduce vascular damage induced by thrombin or to reduce interaction of platelets with plasma clotting factors. No doubt, trials with inhibitors of tumor necrosis factor, IL6 and IL8, as well as elastase will demonstrate additional benefits that have clinical implications.

CONCLUSION

Despite the complexities of the DIC process in the many disorders that give rise to this complication, it has been possible to characterize a mechanism that depends upon thrombin, its activity, and its rate of promotion by tissue factors released from injury of various sorts. Intervention can be offered that controls the hemorrhagic, as well as the thrombotic complications, but usually does not affect the severity or the morbidity resulting from the underlying cause. "Buying time" is perhaps the major attribute of such treatment with the reduction of morbidity and mortality from the DIC complications as a potential benefit. In this regard, heparin has been shown to be effective and should be offered beginning with a prophylactic dose and proceeding to a therapeutic one, as soon as hemostasis is controlled by appropriate replacement of clotting factors.

AT III concentrates hold promise for such management, recognizing that this agent does not appear to increase the bleeding potential, even when remarkably high circulating levels are achieved. Such products can be administered at the first indication of a hypercoagulable state, and if offered in large doses, can be expected to ameliorate the condition. Clinical trials to substantiate this position are underway, and hopefully will demonstrate the efficacy of this approach, whether endotoxin or trauma induced. Other agents may well be found useful to reduce the coagulopathy, which would include antiplatelet agents such as: Aspirin, Dextran 70, and Tyclo-

pidin, as well as some of the newer glycoprotein IIb and IIIa agents under investigation. The newer antithrombins which should be approved shortly, hirudin and its analogue hirulog, and argatroban, for example, may prove particularly beneficial in this condition by eliminating thrombin's damaging effects. On the other hand, substances which block many of the mediators, tumor necrosis factor, IL6 and IL8, as well as elastase, may contribute further to reducing the morbidity associated with the inflammatory aspects of the disorder. In any event, the thrust of our treatment will not change with respect to the need for the earliest intervention possible, and with the large doses of anticoagulants and inflammation inhibitors designed to prevent the explosive progressive characteristic of these disorders.

REFERENCES

1. Schneider CL. Mechanisms of production of acute fibrinogen deficiencies. Prog Hematol 1956;1:202–209
2. Seegers WH. Factors in the control of bleeding. Cincinnati J Med 1950;31:395–342
3. Ratnoff OD, Pritchard JA, Colopy JA. Hemorrhagic states during pregnancy. N Engl J Med 1955;253:63–69
4. Rosenfeld G, Kelen E, NaLas L. Regeneration of fibrinogen after defibrination by bothropic venom in man and dogs. Rev Clin Sao Paulo 1958;34:36–40
5. Owings J, Gosselin M. Acquired antithrombin deficiency following severe traumatic injury. Semin Thromb Hemostas 1997;23 (Suppl):17–24
6. Gando S, Tedo I., Kubota M. Post trauma coagulation and fibrinolysis. Crit Care Med 1992;20:594–600
7. Miller RS, Weatherford DA, Stein D, Crane MM, Stein M. Antithrombin III and trauma patients: factors that determine low levels. J Trauma 1994;37:442–445
8. Lampl L, Helm M, Specht A, Bock KH, Hartel W, Seifried E. Blood coagulation parameters as a prognostic factors in multiple trauma. Zentralbl Chir 1994;119:683–689
9. Nast-Kolb D, Waydhas C, Kerim-Sade C, Jochum M, Spannagl M. Venous thrombosis following severe multiple trauma. Orthopäde 1993;22:110–116
10. Gando S, Nakanishi Y, Tedo I. Cytokines and plasminogen activator inhibitor in post trauma disseminated intravascular coagulation. Crit Care Med 1995;23:1835–1842
11. Kearney TJ, Bentt L, Grode M, Lee S, Hiatt JR, Shabot MM. Coagulopathy and catecholamines in severe head injury. J Trauma 1992;32:608–611
12. Hulka F, Mullins R, Frank E. Blunt brain injury activates the coagulation process. Arch Surg 1996;131:923–927
13. Sorenson JV, Jensen HP, Rahr HB, et al. Haemostatic activation in patients with head injury with and without simultaneous multiple trauma. Scand J Clin Lab Invest 1993;53:659–665
14. Walenga J, Kowal-Vern A, Gamelli R. Laboratory manifestations and other hemostatic disturbances in the traumatized patient. First Int Symp Venous Thromb Grp, Romer Grafik (Den), 1992
15. Neely AN, Warden GD, Rieman M, Friedberg DL, Holder IA. Components of the increased circulating proteolytic activity in pediatric burn patients. J Trauma 1992;33:807–812
16. Saleh AA, Bottoms SF, Farag AM, et al. Markers for endothelial injury, clotting and platelet activation in preeclampsia. Arch Gynecol Obstet 1992;251:105–110
17. Nako Y, Tomomasa T, Morikawa A. Plasma thrombomodulin level in newborn infants with and without perinatal asphyxia. Acta Paediatr 1997;86:91–95
18. Bick R. Disseminated intravascular coagulation. In: Bick RL, ed. Disorders of Thrombosis and Hemostasis. Chicago: ASCP Press; 1992:137–173
19. Pritchard J, MacDonald P, Gant N. Williams. Obstetrics, ed 17, Norwalk, CT: Appleton-Century-Crofts; 1985:8–399
20. Gralnick H, Tan H. Acute promyelocytic leukemia: A model for understanding the role of the malignant cell in hemostasis. Hum Pathol 1974;5:661–673
21. Kario K, Matsuo T, Kodama K, Matsuo M, Yamamoto K, Kobayashi H. Imbalance between thrombin and plasmin activity in disseminated intravascular coagulation. Assessment by the thrombin-antithrombin III complex/plasmin-alpha-2-antiplasmin complex ratio. Haemostasis 1992;22:179–186
22. Lisiewicz J. Disseminated intravascular coagulation in acute leukemia. Semin Thromb Hemostas 1988;14:339–349
23. Rapaport S, Chapman C. Coexistent hypercoagulability and hypofibrinogenemia in a patient with prostatic carcinoma. Am J Med 1959;27:144–153
24. Henry R. Platelet function in hemostasis. In: Murano G, Bick R, eds. Basic Concepts of Hemostasis and Thrombosis. Boca Raton, FL: CRC Press; 1980:17–41
25. Ryan T. Coagulation and fibrinolysis. In: Ryan T, ed. Microvascular Injury. Philadelphia: WB Saunders; 1976:221
26. Bick R. Disseminated intravascular coagulation. Hematol Onco Clin North Am 1992:1259–1285
27. Bick R. Disseminated intravascular coagulation. In: Bick R, ed. Boca Raton, FL: CRC Press, 1983:31–35
28. Bick RL. Hemostasis in liver and renal disease In: Bick RL, ed. Disorders of Thrombosis and Hemostasis. Chicago: ASCP Press; 1992:175–193
29. Baker W. Clinical aspects of disseminated intravascular coagulation. Semin Thromb Hemostas 1989;15:1–57
30. Krevins J, Jackson D, Cowley C, Hurtman R. The nature of the hemorrhagic disorder accompanying hemolytic transfusion reactions in man. Blood 1957;12:834–843
31. Kazemier P, Didisheim P, Fairbanks V, Ludwig J, Payne W, Bowie E. Intravascular coagulation and arterial disease. Thromb Diath Haemorrh 1969;(suppl 36):1969;295–303
32. Schnetzer G, Penner J. Chronic intravascular coagulation syndrome associated with atherosclerotic aortic aneurysm. South Med J 1973;66:264–268
33. Bick R. Clinical review of disseminated intravascular coagulation and related syndromes. Semin Thromb Hemostas 1988; 14:327–338
34. Feinstein D. Treatment of disseminated intravascular coagulation. Semin Thromb Hemostas 1988;14:351–362
35. Larcan A, Lambert H, Gerard A. Consumption Coagulopathies. Paris: Masson; 1987:1–240
36. Penner J. unpublished data. 1972
37. Emerson TE Jr, Fournel MA, Redens TB, Taylor FB Jr. Efficacy of antithrombin III supplementation in animal models of fulminant Escherichia coli endotoxemia or bacteremia. Am J Med 1989;87:27S–33S
38. Blauhut B, Kramer H, Vinazzer H, Bergman N. Substitution of antithrombin III in shock and DIC. Thromb Res 1985;1:81–89
39. Hanada T, Abe T, Takita H. Antithrombin III concentrates for treatment of disseminated intravascular coagulation in children. Am J Ped Hematol Oncol 1985;7:3–8
40. Jochum M. Influence of high dose antithrombin concentrate therapy on the release of cellular proteinases, cytokines, and soluble adhesion molecules in acute inflammation. Semin Hematol 1995;32:4(Suppl 2). 19–32
41. Kowal-Vern A. Antithrombin III concentrates in thermal injury. Submitted for Publication, 1997

Gastroenterologia Japonica
Vol. 24, No. 3
Printed in Japan

—Original Article—

Non-septic endotoxemia in cirrhotic patients

Yoshiaki YAJIMA, Ichiro FUKUDA, Masao OTSUKI, Hiroshi SUZUKI, Kazuo MORI, and Yoshio GOTO
Third Department of Internal Medicine, Tohoku University School of Medicine, Sendai 980, Japan

Summary: We have found that endotoxemia detected by conventional LCT (limulus colorimetric test) in patients with liver diseases could not be detected by endotoxin-specific LCT at all, and proposed that this β-glucan like activity (BGLA) should be termed as non-septic endotoxemia, distinguishing it from septic endotoxemia seen in gram-negative sepsis. In this study, we investigated non-septic endotoxemia through the clinical course of 8 cirrhotic patients. Non-septic endotoxemia appeared at the onset of DIC but tended to decline in level in the late terminal stage. This phenomenon cannot be consistent with the "spillover" theory which explains the mechanism of endotoxemia without sepsis in liver disease. We think it is an urgent problem to elucidate the nature of BGLA in liver disease, without recourse to the "spillover" theory. *Gastroenterol Jpn 1989;24:262–269*

Key words: *β-glucan like activity; DIC; endotoxemia; liver cirrhosis; non-septic endotoxemia*

Intorudction

We previously reported endogenous endotoxemia in liver diseases using the limulus colorimetric text (LCT)[1-3]. Endogenous endotoxemia appeared with the presence of ascites at low levels in some but not in all cases. At the stage of hepatic failure complicated with disseminated intravascular coagulation (DIC) or renal failure, endotoxemia was more frequent and endotoxin concentration greater[2]. Iwanaga et al, however, discovered another pathway activated by β-glucan apart from one by endotoxin in the limulus test and developed the endotoxin-specific LCT[4,5]. We re-evaluated endogenous endotoxemia in liver diseases using the endotoxin-specific LCT and found out that endogenous endotoxemia was not detected by endotoxin-specific LCT at all[3]. Thus far endogenous endotoxin has not been discriminated from septic endotoxin as a chemical substance. They had been separately termed according to their mechanism of appearance in the peripheral blood. We proposed, therefore, to term endogenous endotoxin, which came to be distinguished chemically from septic endotoxin by endotoxin-specific LCT, as non-septic endotoxin[6]. At present, it is not certain whether non-septic endotoxin is a specific type of endotoxin (it may be unresponsive to the endotoxin-specific LCT because of a modification in vivo) or another substance other than endotoxin.

Serial change in non-septic endotoxemia throughout the clinical course of fulminant hepatitis has been previously reported[3]. Non-septic endotoxemia appeared around the onset of DIC and showed a parallel relationship with serum fibrin-degradation products (FDP).

In this study, we report that the same relationship of non-septic endotoxemia and serum FDP as seen in fulminant hepatitis is also present in cirrhotic patients, and discuss the mechanism and the nature of non-septic endotoxemia.

Received November 2, 1988. Accepted November 28, 1988.
Address for correspondence: Yoshiaki Yajima, M.D., Department of Gastroenterology, Sendai City Hospital, Sendai 980, Japan.

Subjects and Methods

Subjects

Eight cirrhotic patients admitted to our hospital were studied. All cases were complicated with DIC in the course of the disease. Of the 8 patients, 6 have died and 2 have survived. The causes of DIC were: spontaneous occurrence from the decompensated state (2), rupture of esophageal varices (2), spontaneous occurrence from the hepatocellular carcinoma-bearing state (1), accompanying complication of acute pancreatitis (1), endoscopic sclerotherapy for esophageal varices (1) and operative reposition of a fractured humerus neck (1).

Methods

Commercially available kits, Toxicolor for the conventional LCT and Endospecy for the endotoxin-specific LCT, were used (both are products of Seikagakukogyo, Tokyo). Heparinized blood samples were treated by the PCA method[7] for deproteinization and subjected to conventional LCT and endotoxin-specific LCT simultaneously. Detection limits of both kits were 5 pg/ml. Details of the PCA-LCT procedure are described elsewhere[2,5].

Results

Endotoxemia in all the cases was detected using conventional LCT. Endotoxin-specific LCT did not detect any endotoxin activity throughout courses of any of the cases. Consequently, endotoxins detected in this study were all non-septic endotoxins according to our definition. Cases are presented as follows.

Case 1. Decompensated liver cirrhosis, 63-year-old male

The patient was admitted for endoscopic sclerotherapy of esophageal varices. Since ultrasonographical examination after admission revealed the presence of a moderate amount of ascites, the prophylactic treatment of varices was postponed. Ascites gradually increased in spite of intensive treatment. On July 1, three months after admission, hepatic encephalopathy and oozing from the injection site appeared. Coagulofibrinolytic data deteriorated as shown by a prothrombin activity of 30% and FDP of 160μg/ml, suggesting the onset of DIC. Hepatic encephalopathy disappeared after a few days but the coagulation disorder deteriorated further in spite of drip infusion of Gabexate mesilate (FOY) and supplementation of coagulation factors. On July 23, the serum bilirubin level began to rise, indicating a state of generalized hepatic failure. About a month after the onset of DIC, the patient died suddenly. Hepatic encephalopathy immediately before death was grade II but this could not explain the cause of the sudden death.

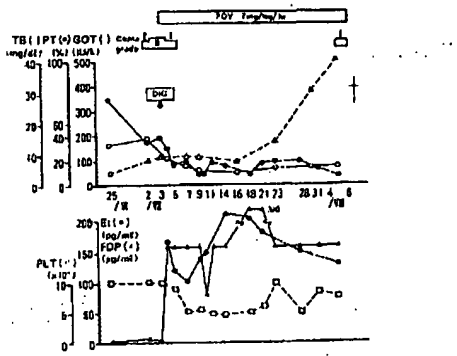

Fig. 1 Clinical course of case 1 (decompensated liver cirrhosis, 63-year-old male).
DIC = disseminated intravascular coagulation, FOY = trade name of Gabexate mesilate, Et = endotoxin.

Measurement of blood endotoxin began immediately after the onset of DIC. The initial endotoxin value was as high as 162pg/ml and high values persisted thereafter. Non-septic endotoxemia exhibited a parallel relationship with the change of serum FDP and tended to decline in level toward the patient's death (**Fig. 1**).

Case 2. Decompensated liver cirrhosis, 58-year-old male

The patient was admitted to our hospital for the prophylactic treatment of esophageal vari-

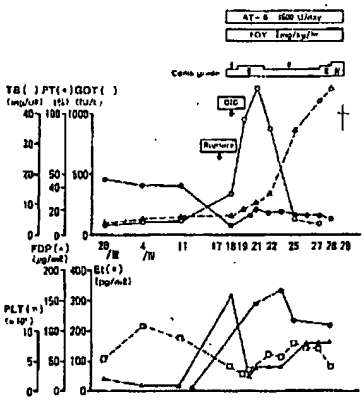

Fig. 2 Clinical course of case 2 (decompensated liver cirrhosis complicated with DIC after variceal rupture, 58-year-old male). Abbreviations are the same as in Figure 1.

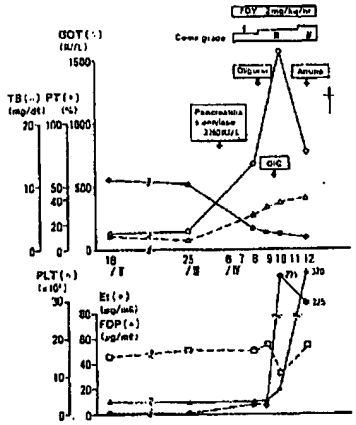

Fig. 3 Clinical course of case 3 (liver cirrhosis complicated with acute pancreatitis, 36-year-old male). Abbreviations are the same as Figure 1.

ces. Ultrasonographical examination revealed the presence of ascites and serum bilirubin was above 3mg/dl, consistent with Child's C group. Therefore, prophylactic therapy was postponed and an improvement of general condition was anticipated. Serum bilirubin, however, remained at a level of 3 to 5 mg/dl and transient encephalopathy was observed. Three months after admission, esophageal varices ruptured. Emergency endoscopic sclerotheray managed to control bleeding, however, DIC developed. Continuous drip of FOY and supplementation of coagulation factors were performed but coagulofibrinolytic data deteriorated further. Jaundice and hepatic encephalopathy became worse and the patient died of generalized hepatic failure.

Non-septic endotoxemia rose after the onset of DIC but tended to decline toward the patient's death (**Fig. 2**).

Case 3. Liver cirrhosis complicated with acute pancreatitis, 36-yeat-old male

The patient, a chronic alcoholic, had suffered from relapsing hepatic encephalopathy. On April 4, he came to our outpatient clinic complaining of upper abdominal pain. Laboratory data showed serum amylase of 3760 IU/L, indicating the complication of acute pancreatitis. He was thus admitted to our hospital on that day. Administration of FOY was begun immediately but the complication of DIC and renal failure could not be avoided, resulting in the patient's death.

Non-septic endotoxemia began to rise simultaneously with the onset of DIC and tended to decline toward the patient's death (**Fig. 3**).

Case 4. Liver cirrhosis complicated with DIC after endoscopic sclerotherapy for esophageal varices, 60-year-old female

The patient was admitted to our hospital for endoscopic sclerotherapy of esophageal varices. Because ascites was present to a slight degree and prothrombin activity was as low as 31%, FOY drip was started prior to sclerotherapy to prevent the onset of DIC. Prothrombin activity, however, decreased significantly on the second day after sclerotherapy. On the fifth day, the elevation of serum FDP was seen, suggesting the complication of DIC. In spite of vigorous treatment, DIC could not be improved and the patient died of cerebral hemorrhage on the tenth day after sclerotherapy.

Non-septic endotoxin could be measured only at 2 points. The first one, measured imme-

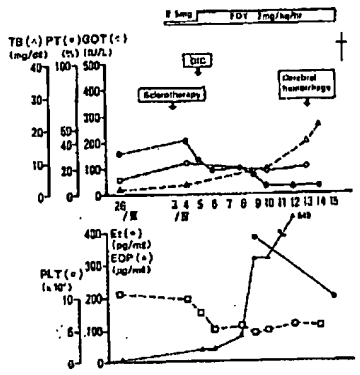

Fig. 4 Clinical course of case 4 (liver cirrhosis complicated with DIC after endoscopic sclerotherapy for esophageal varices, 60-year-old female). Abbreviations are the same as in Figure 1.

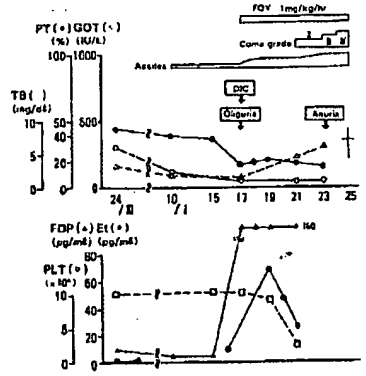

Fig. 5 Clinical course of case 5 (hepatocellular carcinoma with underlying liver cirrhosis, 52-year-old female). Abbreviations are the same as in Figure 1.

diately after the onset of DIC, was extremely high and the second, immediatley before death, was rather low (Fig. 4).

Case 5. Hepatocellular carcinoma with underlying liver cirrhosis, 52-year-old female

The patient was admitted to our hospital for transarterial embolization of a hepatocellular carcinoma. The first embolization was performed on September 26 and the second one was scheduled for the end of the following January. On January 17, however nasal bleeding heralded the onset of DIC, accompanying

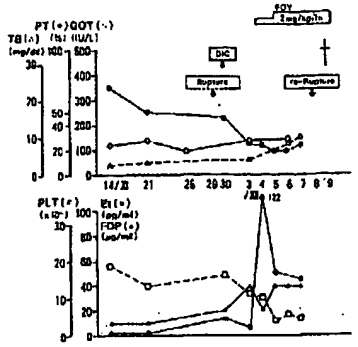

Fig. 6 Clinical course of case 6 (hepatocellular carcinoma with underlying liver cirrhosis, 63-year-old male). Abbreviations are the same as in Figure 1.

simultaneous renal failure. Ascites and hepatic encephalopathy deteriorated further resulting in the patient's death.

Non-septic endotoxemia began to rise slightly after the onset of DIC and tended to decline toward the patient's death (Fig. 5).

Case 6. Hepatocellular carcinoma with underlying liver cirrhosis, 63-year-old male

The patient had been repeatedly admitted to our hospital for treatment of hepatocellular carcinoma and this was his fourth admission. He was scheduled for another TAE but, on November 29, DIC appeared following the rupture of esophageal varices. With endoscopic sclerotherapy, bleeding was halted but DIC could not be improved. On the tenth day after the first rupture, the patient died of re-rupture of varices.

Non-septic endotoxemia rose with a time lag after the onset of DIC and tended to decline toward the patient's death (Fig. 6).

Case 7. Decompensated liver cirrhosis complicated with DIC, survived, 59-year-old female

The patient was admitted to our hospital for the fourth time because of massive asctes and increasing jaundice, suggesting severe decompensated liver cirrhosis. On the third hospital

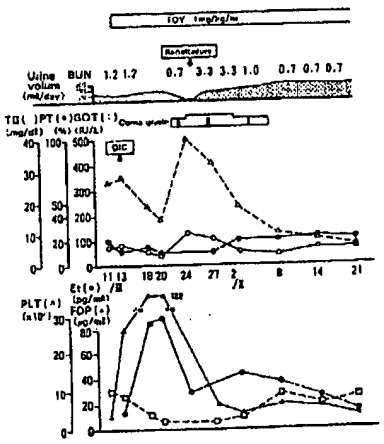

Fig. 7 Clinical course of case 7 (decompensated liver cirrhosis complicated with DIC, alive, 59-year-old female). Abbreviations are the same as in Figure 1.

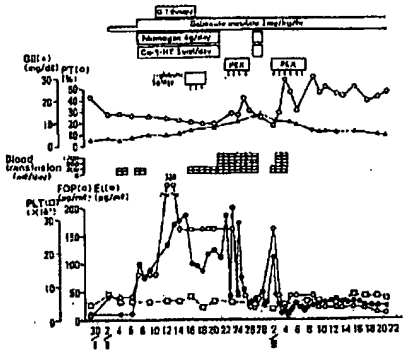

Fig. 8 Clinical course of case 8 (liver cirrhosis complicated with DIC after surgical reposition of humerus under general anesthesia, alive, 61-year-old female). Abbreviations are the same as in Figure 1.

day, coagulofibrinolytic data deteriorated to the onset of DIC, accompanying renal failure. Continuous drip infusion of FOY was found to be successful in managing DIC, followed by the restoration of renal function.

A proportional relationship was observed between changes in non-septic endotoxin and serum FDP throughout the course (Fig. 7).

Case 8. Liver cirrhosis complicated with DIC after surgical reposition of humerus under general anesthesia, survived, 61-year-old female

The patient underwent surgical repositioning of fractured humerus under general anesthesia. After an uneventful operation, however, ascites appeared and worsened. She was transferred to our department for control of the ascites. Though the asites improved gradually, coagulofibrinolytic data deteriorated to an FDP of 40μg/ml and PT activity of 28%, indicating the onset of DIC. In spite of the drip infusion of FOY and supplementation of coagulation factors, coagulofibrinolytic data further deteriorated as shown by an FDP of 320μg/ml. Bleeding from the operative wound began and the volume reached ca 1 L/day. The decision was made to start plasma exchange to control the massive bleeding.

Plasma exchange started from Feb 22 and continued for 4 consecutive days, with an exchange volume of 3-5 L/day. Coagulofibrinolytic data improved remarkably as shown by PT activity of 40% and FDP of 20μg/ml, while the bleeding from the operative site was scarcely visible. On March 2, coagulation data deteriorated and the bleeding started again. After another series of plasma exchanges, on 6 consecutive days, coagulofibrinolytic data were maintained as follows: FDP 20μg/ml, PT activity 40-50%, fibrinogen 100-120%. With the improvement of coagulofibrinolytic data, bleeding from the operative site decreased gradually and finally stopped.

On admission, when massive ascites was present, the non-septic endotoxin level was 11pg/ml. It then rose to 100pg/ml on February 7 when serum FDP reached the level of 80μg/ml. Thereafter, non-septic endotoxin shifted in a parallel relationship with serum FDP. After beginning plasma exchange, non-septic endotoxin values tended to decrease after plasma exchange and to increase again the following morning. Thus, the change in non-septic endotoxin exhibited a serrated curve. The third plasma exchange, however, was not followed by a rise in non-septic endotoxin the following

morning. Interruption of the plasma exchange worsened the coagulofibrinolytic data and allowed a rise in non-septic endotoxemia which lagged behind the rise of serum FDP. After the recovery from DIC, non-septic endotoxemia of around 20 pg/ml persisted (Fig. 8).

Discussion

In 1972, Caridis et al[8] reported 2 cases of liver cirrhosis in which endotoxin was detected by the limulus assay without bacteremia. This was the first report concerning endogenous endotoxemia. In 1976. Wilkinson et al[9] reported 45 cases of cirrhotics in which endogenous endotoxemia was detected in 84% of those with renal failure and in 10% of those without renal failure. Clemente et al (1977)[10] also reported 43 cases of cirrhotics with ascites in which endotoxemia was detected in 80% of those with renal failure and in 3% of those without. On the other hand, Tarao et al (1977)[11] and Liehr et al (1977)[12] reported that endotoxemia was detected in 70-80% of cirrhotics with ascites, with almost the same percentage in their ascites and even in some 20% of cirrhotics without ascites. Fulenwider et al (1980)[13] could not substantiate these findings but reported that when the limulus gelation test was performed on the peripheral plasma of 38 cirrhotic patients, portal plasma and ascites in 14 and 11 of these patients, respectively, no endotoxin was detectable.

According to our results[2] using limulus colorimetric test after treatment with the PCA method (PCA-LCT), endotoxin was not detectable at all in cirrhotic patients without ascites and in 52% of those with ascites. At the stage of hepatic failure complicated with renal failure or DIC, endotoxin was more frequent and endotoxin concentration greater. Consequently, our results support the results of Wilkinson et al or Clemente et al and conform to the results of Fulenwider et al with respect to the undetectability of endotoxemia in cirrhotic patients without ascites. Concerning the detection ratio of endotoxemia in cirrhotic patients with ascites, Fulenwider et al reported 0%, Wilkinson et al 10%, Clemente et al 3%. These data were far lower than our own result of 52%. This discrepancy can be explained by the difference in detection limits of the limulus gelation test (LGT) and LCT. Our investiation showed the detection limit of LGT to be 20-40 pg/ml[14]. Most of the endotoxin levels detected in cirrhotic patients with ascites were less than 10 pg/ml, which is below the detection limit of LGT. We consider that the ubiquity of endotoxin, with the attendant opportunities for specimen contamination, is the most likely explanation of the high prevalence of endotoxin in the plasma and ascites reported by Tarao et al and Liehr et al. Fulenwider et al reported that the samples that yielded positive results were immediately retested in duplicate and were found to be negative[13]. Thus, the data obtained with LCT conform to those obtained with LGT. This conformity is reasonable if we consider that both are bioassays sharing a common basis, limulus lysate.

In this study in which serial changes in endotoxin were documented in each cirrhotic patient using LCT, a close relationship between the appearance of endotoxemia and the onset of DIC was observed as a parallel relationship between changes of non-septic endotoxemia and serum FDP. We have already reported this parallel relationship in patients with fulminant hepatitis in whom the change in non-septic endotoxemia was influenced by plasma exchange[3]. In cirrhotic patients in whom plasma exchange was usually not performed, the natural progress of non-septic endotoxemia could be seen. Based on the "spillover" theory, it is conceivable that endotoxemia exacerbates toward patients' death because of the acceleration of the spillover of endotoxin through the failing liver. In this study, however, non-septic endotoxin tended to decline toward patients' death. This phenomenon cannot be explain by the "spillover" theory.

We have previously reported[3] that endogenous endotoxemia seen in liver diseases could not be detected by the endotoxin-specific LCT developed by Iwanaga et al. For that reason, we proposed to term endogenous endo-

toxin as non-septic endotoxin to stress the difference between endogenous endotoxin and septic endotoxin on a physical basis. Non-septic endotoxemia appears in the operation for esophageal carcinoma[15] or in hepatectomy for cirrhotic livers[16] without complications of shock or DIC. We doubted that non-septic endotoxin had biological activity as a toxin. At that point, however, the possibility could not be ruled out that endotoxin was modified during the course of spillover, suffering some structural change. Though admitting this possibility, the tendency of non-septic endotoxin to decline close to death cannot be explained by the "spillover" theory and non-septic endotoxin cannot be understood as a spilled endotoxin any longer. Hereafter, we propose to term non-septic endotoxin as β-glucan like activity (BGLA), from the point of view of describing non-septic endotoxin as a unique phenomenon on limulus assay, apart from the "spillover" theory.

Even if the positive reaction of the limulus assay seen in liver disease is duo to BGLA, it does not conflict with the "spillover" theory. A problem was pointed out with the PCA method, a widely prevalent pretreatment for LCT. As we have previously reported[17], when standard endotoxin was added to normal plasma and the mixture was incubated at 37°C, the recovery of added endotoxin decreased rapidly to a level of 10^{-3} -10^{-4} of the initial concentration within 20-30 minutes, if PCA-LCT were used. However, this residual endotoxin was found to be stable in the plasma, even in fresh plasma. The endotoxin detected in patients with gram negative septicemia was also found to be stable both in the patients own plasma and in the plasma of normal controls. Takahashi et al[18] supposed that added endotoxin combined with some kind of protein and PCA treatment precipitated the complex. They reported recovery from the precipitate of the total amount of added endotoxin. From these facts, the following hypothesis can be presented: When entering into the blood stream, endotoxin rapidly combines with a carrier protein (perhaps a high-density liporotein[19] but a minute portion of it remains free. When the PCA method is used as a pretreatment, only the free endotoxin is detectable because, in PCA method, only the supernate after deproteinization is available for LCT. Takahashi et al have further progressed to improve the PCA method and developed a new PCA method[18] which can detect protein-binding endotoxin as well. We evaluated this new PCA method and found that it recovered almost 100% of added endotoxin when the conventional PCA method recovered none of it (unpublished data). Estimating from the in vitro results, the amount of protein-binding endotoxin totals 10^{3} -10^{4} of that of the free endotoxin. Therefore, for the study of septic endotoxin, we should use the new PCA method and the "spillover" theory should be substantiated using the new PCA method. With respect to BGLA, its nature remains unknown and it is uncertain which pretreatment is more desirable. We think we may rationally progress in our study of BGLA using the PCA method for the time being.

References

1. Yajima Y, Otsuki M, Suzuki H, et al: Endotoxemia in various liver diseases and gram-negative septicemia using limulus colorimetric test (LCT). Jpn J Gastroenterol 1984;81:2538-2543 (In Jpn)
2. Yajima Y, Fukuda I, Otsuki M, et al: Endotoxemia in liver diseases: Detection by a quantitative assay using chromogenic substrate with perchloric acid pretreatment. Tohoku J Exp Med 1985;147:411-419
3. Yajima Y, Fukuda I, Otsuki M, et al: Re-evaluation of endotoxemia in liver diseases using Endotoxin-Specific (E-S) limulus colorimetric test. The 7th proceeding of the congress of endotoxin clinical research. Yotosha, Tokyo. 1987;47-54 (in Jpn)
4. Iwanaga S, Morita T, Miyata T, et al: The limulus coagulation system sensitive to bacterial endotoxins. In: Homma JY, et al, eds. Bacterial endotoxin: Chemical, biological and clinical aspects. Verlag Chemie, Weinheim 1984;365-382
5. Obayashi T, Tamura H, Tanaka S, et al: A new chromogenic endotoxin-specific assay using recombined limulus coagulation enzymes and its clinical applications. Clin Chim Acta 1985;149:55-65
6. Yajima Y: Biological activity of endotoxin and clinical significance of endotoxemia. Prog Med 1987;7:1085-1094 (In Jpn)
7. Tamura H, Obayashi T, Takagi T, et al: Perchloric acid treatment of human blood for quantitative endotoxin assay using synthetic chromogenic substrate for horseshoe crab clotting

enzyme. Thromb Res; 1982;27:51-57
8. Caridis DT, Reinhold RB, Woodruff PWH, et al: Endotoxemia in man. Lancet 1972;1:1381-1386
9. Wilkinson SP, Moodie H, Stamatakis JD, et al: Endotoxemia and renal failure in cirrhosis and obstructive jaundice. Br Med J 1976;2:1415-1418
10. Clemente C, Bosch J, Rodes J, et al: Funcional renal failure and haemorrhagic gastritis associated with endotoxemia in cirrhosis. Gut 1977;18:556-560
11. Tarao K, So K, Moroi T, et al;; Detection of endotoxin in plasma and ascitic fluid of patients with liver cirrhosis: Its clinical significance. Gastroenterology 1977;73:539-542
12. Liehr H, Grun M: Clinical aspects of Kupffer cells failure in liver disease. In: Wisse E, Knock DL, eds. Kupffer cells and other sinusoidal cells. Elsevier Publishing Co, Amsterdam-London-New York 1977;427-436
13. Fulenwider JT, Sibley C, Stein SF, et al: Endotoxemia of Cirrhosis: An observation not substantiated. Gastroenterology 1980;78:1001-1004
14. Yajima Y, Fukuda I, Otsuki M, et al: Comparison between limulus gelation test (LGT) and limulus colorimetric test (LCT). The 6th proceedings of the congress of endotoxin clinical research. Yotosha, Tokyo, 1985;47:53 (in Jpn)
15. Kitamura M, Nishihira T, Hirayama K, et al: Postoperative endotoxemia in patients with esophageal carcinoma. Jpn J Gastroenterol Surg. 1987;20:1648-1653 (in Jpn)
16. Nakagawa K, Matsubara S, Ouchi K, et al: Endotoxemia after abdominal surgery. Tohoku J Exp Med 1986;150:273-280
17. Yajima Y, Fukuda I, Otsuki M, et al: Stability of endotoxin detected in human plasma against endotoxin-inactivating factor (EIF): Quantitative analysis of EIF using chromogenic endotoxin assay Tohoku J Exp 1986;150:317-327
18. Takahashi K: Study on quantitative measurement of endotoxin in human blood using chromogenic substrate: Especially pretreatment of plasma. J Iwate med Ass 1988;40:67-81 (in Jpn)
19. Freudenberg MA, Bog-Hansen TC, Back U, et al: Interaction of lipopolysaccharides with plasma high-density lipoprotein in rats. Infect Immun 1980;28:373-380